Claims

1. A method of identifying organisms by comparative genetic analysis, characterized in that coding and/or non-coding areas and/or functionally significant areas of highly conserved genes and/or their homologous genes and/or their cDNA copies and/or their pseudogenes are amplified using PCR and are subsequently genotyped and analyzed.

2. The method according to claim 1, characterized in that one primer pair each is used for each specific segment of the highly conserved gene, which is located in the highly conserved exon region and/or non-coding areas and/or functionally significant areas and/or in the 5'- or 3'-untranslated area of the gene and binds in as many studied species DNAs as possible, preferable in all studied species DNAs, and enables the amplification of the corresponding gene area.

3. The method according to claims 1 and 2, characterized in that coding and/or non-coding areas located between the primers and being either highly variant intron regions and/or variant exon regions or 5'- or 3'-untranslated areas of the gene, are analyzed as regards their sequence and identified by comparison with the species-specific sequence variants.

4. The method according to claims 1 to 3, characterized in that either the sense strand or the antisense strand of any species DNA or also their PCR copies are used for the identification.

5. The method according to claims 1 to 4, characterized in that preferably animals are identified.

6. The method according to claims 1 to 4, characterized in that preferably vertebrates are identified.

7. The method according to claims 1 to 4, characterized in

that preferably mammals are identified.

8. The method according to claims 1 to 4, characterized in that preferably plants are identified.

9. The method according to claims 1 to 4, characterized in that genotying is carried out by DNA sequencing, any hybridization methods, restriction fragment length analyses, chromatographic methods, spectroscopic and in particular mass-spectroscopic methods, allele-specific PCR or by other methods suitable for detecting DNA sequence variants.

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10. The method according to claims 1 to 4, characterized in that exon and/or intron areas as well as functionally significant areas of the highly conserved tumor suppressor gene PTEN/MMAC1 and its homologues are used for amplification and subsequent genetic analysis.

11. The method according to claims 1 to 4, wherein cDNA copies of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

12. The method according to claims 1 to 4, wherein pseudogenes or segments of pseudogenes of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

13. The method according to claims 1 to 4, characterized in that preferably exons arranged side by side of the PTEN/MMAC1 gene and its homologues and/or the parts of the introns following the exons are analyzed genetically.

14. The method according to claims 1 to 4, characterized in that the exon regions 1 and 2 and/or 3 and 4 and/or 4 and 5 and/or 5 and 6 and/or 6 and 7 and/or 7 and 8 and/or 8 and 9 with the enclosed intron regions 1 and/or 2 and/or 3 and/or 4 and/or 5 and/or 6 and/or 7 and/or 8

as well as the 5'- and 3'-untranslated regions of the PTEN/MMAC1 gene and their homologues are used for the genetic analysis.

15. The method according to claims 1 to 4, characterized by selecting areas of highly conserved genes and/or pseudogenes and their homologues, constructing suitable oligonucleotides as primers which bind to the corresponding complementary coding and/or non-coding areas and/or functionally significant areas, amplifying them by means of a suitable technique and comparatively analyzing the sequence of the corresponding coding and/or non-coding area of various species by genetic analysis.

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16. The method according to claim 15, characterized in that areas of the PTEN/MMAC1 gene and/or the pseudogene and their homologues are selected.

17. The method according to claims 15 and 16, characterized in that differing sequence segments of each individual exon, intron or untranslated region of the PTEN/MMAC1 gene and their homologues or the corresponding cDNA are selected.

18. The method according to claims 1 to 17, characterized in that genotyping of pig DNA which is obtained preferably from foodstuffs, is carried out on the basis of the gene sequence variant of PTEN/MMAC1 containing a 9-base pair long deletion.

19. An oligonucleotide primer for the PCR and the sequencing of exon 1 and/or 5'-untranslated region of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex1-401 sense
5'-cccttctactgcctcca -3'

PTENex1 -465 sense
5'- gggaggggtctgagt -3'

PTENex1 ATG sense
5'- atgacagccatcatcaaaga -3'

PTENex1 R antisense
5'- aggtcaagtctaagtcgaatc -3'

20. The oligonucleotide primer for PCR and the sequencing of exon 2 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

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PTENex2F sense
5'- atatttatccaaacattattgctat -3'

PTENex2R antisense
5'- cttactacatcatcaatattgttcc -3'

21. The oligonucleotide primer for PCR and the sequencing of exon 4, intron 4 and exon 5 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

Zoo43sUV sense
5'- tgtgctgagagacattatgac -3'

SPL5 sense
5'- aaatttaattgcagaggt -3'

Zoo44aRV antisense
5'- ttgtctctggtccttacttc -3'

22. The oligonucleotide primer for PCR and the sequencing of exon 5 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTEN se sense
5'- atcttgaccaatggctaagtg -3'

09936238.091701

Zoo44aRV antisense
5'- ttgtctctggtccttacttc -3'

23. The oligonucleotide primer for PCR and the sequencing of exon 6 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex6F sense
5'- gga gta act att ccc agt cag ag -3'

PTENex6R antisense
5'- gca agt tcc gcc act gaa -3'

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24. The oligonucleotide primer for PCR and the sequencing of exon 7 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex7F sense
5'- cct cag ttt gtg gtc tgc ca -3'

PTENex7R antisense
5'- c ctt ttt tag cat ctt gtt ctg ttt -3'

25. The oligonucleotide primer for PCR and the sequencing of exon 8 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex8F sense
5'- caa aat gtt tca ctt ttg ggt aaa -3'

PTENex8R antisense
5'- taa aat ttg gag aaa agt atc ggt t -3'

26. The oligonucleotide primer for PCR and the sequencing of exon 9 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex9F sense

5'- gtg aag ctg tac ttc aca aaa ac -3'

PTENex9tga antisense
5'- aaa aaa att cag act ttt gta att tg -3'

27. The method according to claims 1 to 17, characterized in that for the DNA amplification a mixture of oligonucleotides is used which differ at the 3' region of the oligonucleotide as regards its length by one or more nucleotides or which differ as regards their nucleotide sequence at the 3' end of the oligonucleotide at one or more positions.

28. The method according to claims 1 to 17 and 26, wherein the oligonucleotides

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sense:
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat -3',
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t -3',
antisense:
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',
5'- cag gaa aca gct atg act tgt ctc tgg tcc t -3'

are used for the amplfication.

29. The method according to claims 1 to 17 and 26, wherein the oligonucleotides

sense:
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gaa -3',
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gag -3',
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gat -3',
antisense:
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt a -3',
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt g -3',
5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt t -3'

are used for the amplification.

30. The method according to claims 1 to 17, characterized in that DNA sequencing methods are used for the genetic analysis.

31. The method according to claims 1 to 17, characterized in that DNA sequencing techniques are used in the genetic analysis for the PTEN/MMAC1 and/or its pseudogenes and their homologues.

32. The method of distinguishing the DNA of various species, characterized in that at least one hybridization probe pair is used, the melting points of different combinations are determined and compiled for each species into a panel.

33. The method of distinguishing the DNA of various species, characterized in that at least one hybridization probe pair is used and at least one gene segment is amplified, differing hybridization probe pairs hybridize to different gene segments, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

34. The method of distinguishing the DNA of different species according to claim 33, characterized in that at least one hybridization probe pair is used and at least one gene segment of at least one species is amplified, differing hybridization probe pairs hybridize to different gene segments of various species, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

35. The method of distinguishing the DNA of various species according to claims 33 and 34, characterized in that at least two hybridization probes of SEQ Nos. 3 to 8 are used, the melting points of different combinations are determined and compiled for each species into a panel.

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36. The method according to claims 33 and 34, characterized in that the species differentiation of pig DNA from various other species is made using the hybridization probe pair A1/A2 as the hybridization probe pair.

37. The method according to claims 33 and 34, characterized in that the hybridization probes are used in combinations C1/C2; A1/B2; A1/A2; C1/A2; B1/B2; B1/A2 for the species differentiation between various species.

38. LightCycler hybridization probes for exon 5, characterized by the sequences:

A1: 5'- tgc ata ttt gtt tca tcc ggg caa att - fluorescein -3'
A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph -3'
B1: 5'- tgc ata ttt att aca tcg ggg caa att - fluorescein -3'
B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -3'
C2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

39. LightCycler hybridization probes for exon 6, characterized by the sequences

PTENex6FL
5'- tca tct gga tta tag acc agt ggc act - fluorescein -3'

PTENex6LC 640
5'- LC Red 640 - ttc aca aga tga tgt ttg aaa cta ttc caa- ph -3'

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PTENex6F*
5'- gtg cca ctg gtc tat aat cca gat- fluorescein -3'

PTENex6L* 705
5'- LC Red 705- ttc ttt aac agg tag cta taa taa tac aca ta- ph -3'

40. The LightCycler hybridization probes for exon 7, characterized by the sequences

PTENex7F*
5'- taa agg tga aga tat att cct cca att ca - fluorescein -3'

PTENex7L*640
5'-LC Red 640- acc cac acg acg gga aga caa g - ph -3'

PTENex7 FL
5'-ggtaacggctgagggaactcaaagtac - fluorescein -3'

PTENex7 LC (705-labeled)
5'-LC Red 705- tgaacttgtcttcccgtcgtgtgg- ph -3'

41. The LightCycler hybridization probes for exon 8, characterized by the following sequences

PTENex8F*
5'- tga caa gga ata tct agt act tac ttt aac aaa- fluorescein -3'

PPTENex8L* 705
5'-LC Red 705 - ctt gac aaa gca aat aaa gac aaa gc- ph - 3'

PTENex8 FLU
5'- tgctatcgatttcttgatcacatagacttccatttt - fluorescein - 3'

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PTENex8 LCR (640-labeled)
5'-LC Red 640- actttttctgaggtttcctctggtcctggtat - ph -3'

42. The LightCycler hybridization probes for exon 9, characterized by the following sequences

PTENex9 FL
5'-aac atc tgg tgt tac aga agt tga act gct- fluorescein -3'

PTENex9 LC 640
5'-LC-640- cct ctg gat ttg acg gct cct cta ct - ph -3'

43. Hybridization probe pair A1/A2: specific to PTEN pseudogene pig, characterized by

SEQ No. 3 A1: 5'- tgc ata ttt gtt tca tcc ggg caa att - fluorescein -3'

SEQ No. 4 A2: 5'-LC Red 705- tta aag gca caa gat ttc tat ggg ga - ph -3'

44. Hybridization probe pair B1/B2: specific to pseudogene man, characterized by

SEQ No. 5 B1: 5'- tgc ata ttt att aca tcg ggg caa att - fluorescein -3'

SEQ No. 6 B2: 5'-LC Red 640- aag gca caa gag gcc cta gat ttc ta -ph -3'

45. Hybridization probe pair C1/C2: specific to PTEN pseudogene man (C2) and homologue of pig (C1), characterized by

SEQ No. 7 - C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -3'

SEQ No. 8 - C2: corresponds to probe B2.

46. DNA sequences and/or fragments of homologues of the PTEN/MMAC1 gene and/or of the homologues of the PTEN/MMAC1 pseudogene, which code for proteins involved in the cell-cell adhesion and cell cycle regulation and have an indispensable function in embryogenesis of the respective species and which are compiled in the annex under "List of species sequences".

47. DNA sequences of homologues of the PTEN/MMAC1 gene and/or of homologues of the PTEN/MMAC1 pseudogene, which are compiled in the annex under "list of species sequences", which as compared to the PTEN/MMAC1 gene and/or the PTEN/MMAC1 pseudogene comprise genetic variants such as base substitutions and/or insertions and/or deletions and are suited for identifying corresponding species.

48. A kit for carrying out the method according to claims 1 to 18 and further claims, comprising:

a) one or more vessels comprising PCR and/or sequencing oligonucleotides binding to highly conserved genes, the oligonucleotides being optionally labeled radioactively or by means of a

dye or in another way,

b) vessels having further common reagents for DNA amplification and/or DNA analysis, in particular for DNA sequencing,

and

c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

49. The kit according to claim 48 for carrying out the method according to claims 1 to 18 and further claims, comprising:

a) one or more vessels with PCR and/or sequencing oligonucleotides according to claims 19 to 26.

SUB A2 cont.

50. Kit for identifying species for carrying out the method according to claims 1 to 18 and further claims, comprising:

a) a vessel having an oligonucleotide pair comprising the following sequences:
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3' and 5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

b) two vessels with one of the following sequencing oligonucleotides each, these oligonucleotides being optionally labeled radioactively or by means of a dye or in another way:
5'- cag gaa aca gct atg ac -3' and
5'- cga cgt tgt aaa acg acg gcc agt -3',

c) a vessel containing a control DNA, which is suited for testing the oligonucleotides and the reaction conditions.

51. The kit (Light Cycler Kit) for carrying out the method according to claims 32 to 37 and further claims, comprising

a) one or more vessels containing PCR primers and hybridization probes, which bind to highly conserved genes, the hybridization probes being

optionally labeled by means of a dye,

b) vessels containing further common reagents for DNA amplification and/or DNA analysis, in particular for the Light Cycler Analyses,

and

c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

SUB A2 cont.

52. The kit (Light Cycler Kit) for carrying out the method according to claims 32 to 37 and further claims, comprising:

a) one or more vessels with PCR primers and hybridization probes according to claims 38 to 42.